Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Do Not Enter AOS

1. (Currently amended) A method for detection of an antibody against a pathogenic organism in a liquid sample, wherein said pathogenic organism is selected from the group consisting of bacteria, viruses and protozoa, the method comprising

a) incubating

(1) said sample,

(2) a solid phase,

(3) a first antigen for said antibody, wherein the first antigen has at least one marker group, and comprises multiple epitope regions, said epitope regions being identical in amino acid sequence and

(4) a second antigen for said antibody, wherein the second antigen binds to the solid phase,

under conditions to obtain a complex comprising a solid phase-bound second antigen to which is bound the antibody and to which is bound the first antigen; and

b) detecting said antibody by direct or indirect detection of the marker group on said solid phase; and

wherein at least ~~one of~~ said first antigen is of formula (Ia) or (Ib)

$(P\text{-})_nT(\text{-}L)_n$, (Ia)

$T(\text{-}P\text{-}L_m)_n$ (Ib)

wherein

T is a carrier,

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Detection of Antibody IgG to HIV-1 in Urine by Ultrasensitive Enzyme Immunoassay (Immune Complex Transfer Enzyme Immunoassay) Using Recombinant p24 as Antigen for Diagnosis of HIV-1 Infection

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Anti-HIV-1 IgG in urine was detected by an ultrasensitive enzyme immunoassay (immune complex transfer enzyme immunoassay) using recombinant p24 *gag* protein (p24) of HIV-1 as antigen and β-D-galactosidase from *Escherichia coli* as label. Anti-HIV-1 IgG in urine was reacted simultaneously with 2,4-dinitrophenyl-bovine serum albumin-recombinant p24 conjugate and recombinant p24-β-D-galactosidase conjugate. The complex formed, consisting of the three components, was trapped onto polystyrene balls coated with affinity-purified (anti-2,4-dinitrophenyl group) IgG, eluted with εN-2,4-dinitrophenyl-L-lysine, and transferred to polystyrene balls coated with affinity-purified (anti-human IgG γ-chain) IgG. Bound β-D-galactosidase activity was assayed by fluorometry. This assay was at least 3,000-fold more sensitive than conventional methods. The lowest signal among 49 asymptomatic carriers was 3.1-fold higher than the highest nonspecific signal among 100 seronegative subjects. The sensitivity and specificity were both 100%. The positivity could be confirmed by preincubation of urine samples with excess of the antigen. Thus, this assay would be a powerful tool for detecting IgG antibody to HIV-1 in urine.

Key words: antibody, human immunodeficiency virus type 1, p24, β-D-galactosidase, ELISA, gelatin particle agglutination

INTRODUCTION

Antibodies to human immunodeficiency virus type 1 (HIV-1) in serum, plasma and whole blood have been detected by various methods such as enzyme-linked immunosorbent assay (ELISA), agglutination of latex, red-cells and gelatin particles, dot blotting, and Western blotting (1). Antigens used are the whole virus, recombinant proteins, and synthetic peptides (1). These tests are sufficiently sensitive for the diagnosis of HIV-1 infection (1). However, blood should be collected with due cautions to avoid infections not only with HIV but also with other pathogens. By contrast, urine can be collected more easily with no invasive procedures, less expenses, and less possibility of various infections than blood (2). Recently, a sensitive enzyme immunoassay (immune complex transfer enzyme immunoassay) using recombinant reverse transcriptase (RT) and p17 of HIV-1 (NL4-3) (3) as antigens and horseradish peroxidase and *Escherichia coli* β-D-galactosidase, respectively, as labels has been described for the detection of antibody IgG to HIV-1 in urine (4). This assay was at least 30-fold more sensitive than conventional methods. The sensitivity and specificity were both 100%. The positivity with one of the two antigens could be confirmed by using the other antigen and/or approximately 10-fold concentrated urine samples. Test results were considered to become more reliable by increasing the number of antigens such as RT and p17, with which the sensitivity and specificity are both 100%.

This paper describes the detection of anti-HIV-1 IgG in urine by the immune complex transfer enzyme immunoassay

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